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Antisperm Autoantibody Response After Unilateral Vas Deferens Ligation in Rats: When Does it Develop?

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ABSTRACT: Unilateral obstruction or injury to the vas deferens can result in significant injury to the contralateral testicle. Antisperm autoantibodies are thought to play a significant role in this phenomenon. It has been reported that early surgical repair of the vas, before the development of antisperm autoantibodies, will prevent any potential damage to the contralateral testicle. This led us to investigate the timing of the antisperm antibody production and to attempt to determine whether antibody production precedes histologic testicular damage in the Lewis rat model. In a controlled study, mature rats were divided into temporal groups, with the experimental animals all receiving a unilateral vasectomy. At postoperative endpoints of 1, 7, 15, or 30 days, blood samples were collected for immunologic assay, and the testicles were harvested for histologic examination. Antibody levels were measured by an immunobead test using goat anti-rat immunoglobulin G (IgG)-coated Sepharose beads; tissue sections were fixed in Bouin solution, embedded in paraffin, and stained with hematoxylin and eosin. There was no statistically significant histologic difference between any of the groups. However, immunologic evaluation revealed a statistically significant increase in immunobead antibody binding in the 30-day group compared to the control groups ($P = .02$). These data seem to indicate that in this model, antisperm antibody production is not evident until 15–30 days after unilateral injury to the vas deferens occurs, and the development of these antibodies precedes any demonstrable histologic damage to the testicle. If it is correct to infer that human antisperm antibody production will also precede histologic testicular damage, and further, that the onset of the human autoantibody response may vary from several days to weeks, then in cases of suspected or known ductal injury, the clinical monitoring of antisperm antibody levels could enable testicular damage to be predicted prior to its development and thus be avoided.

Key words: Autoimmune response, vasectomy, testicular damage, immunologic infertility.

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Unilateral obstruction of the vas deferens can occur for a variety of reasons, including epididymitis, trauma, or iatrogenic damage to the vas during hydrocele repair, orchidopexy, or inguinal herniorrhaphy (Lynn and Johnson, 1961; Sparkman, 1962; Shandling and Janik, 1981). Previous studies have revealed that unilateral injury to the vas deferens can result in significant injury to the contralateral testicle (Chehval et al, 1995), and although studies have indicated that the damage can be reversed by repair of the obstructed vas deferens (Matsuda et al, 1992), other studies have suggested that the effects are irreversible (West et al, 2000).

The effect of contralateral testicular damage after unilateral vas ligation has been demonstrated to be mediated through antisperm autoantibodies in several studies (Haas, 1987; Pedersen et al, 1987; Flickinger et al, 1988; Chehval et al, 1995). It has been reported that early repair of the severed vas, before the development of antisperm autoantibodies, will avoid potential damage to the contralateral testicle (Flickinger et al, 2000). In the present study, we have addressed the timing of antisperm antibody production, and we attempt to determine whether the onset of antibody production precedes histologic testicular damage.

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Materials and Methods

Animals

A total of 50 mature (Baker et al, 1979) male Lewis rats, 60–61 days old (200–250 g), were used in this experiment. They were housed in polycarbonate cages in groups of 2–3 animals, had free access to water, and were fed LabDiet 5001 Rodent Diet (PMI Nutrition International Inc, Brentwood, Mo) ad libitum. They were maintained in a room with a 12-hour light:dark cycle and a constant temperature of 22°C. All operative procedures and blood sampling were conducted under halothane inhalation anesthesia. The surgical procedure was performed through a groin incision. After the left vas deferens was exposed, hemoclips were applied approximately 5 mm apart, and then the vas was transected between these clips using scissors. The 2 hemoclips were left in

Table 1. *Immunobead test % binding* (*continuous*)*

Sample	Mean ± SD	*P* Value
Control (n = 10)	40.3 ± 16.7	
1 day (n = 10)	49.2 ± 13.6	.21
7 days (n = 10)	44.7 ± 11.3	.50
15 days (n = 10)	45.8 ± 14.3	.44
30 days (n = 9)	56.4 ± 9.5	.02†

* Means and standard deviations are reported for each sample. (Two determinations per specimen from the immunobead test were averaged for use in the analysis.)
† Clinically significant.

Table 2. *Immunobead test % binding* (*categorized*)*

	Bead Test Result		
Sample	Normal	Positive	*P* Value
Control (n = 10)	5 (50%)	5 (50%)	
1 day (n = 10)	2 (20%)	8 (80%)	.35
7 days (n = 10)	5 (50%)	5 (50%)	1.00
15 days (n = 10)	4 (40%)	6 (60%)	1.00
30 days (n = 9)	0	9 (100%)	.03†

* Percentage binding was categorized into 2 groups: 1) greater than 40% binding is a positive result, and 2) 40% or less binding is negative. Proportions of specimens in each experimental group with positive and negative results were compared with the control sample. (The 2 determinations per specimen from the immunobead test were averaged for use in the analysis.)
† Clinically significant.

place, thereby occluding both cut ends. The incision was closed in multiple layers with resorbable suture, and the animal was monitored until fully recovered. At the end of each test interval, the animals were euthanized by CO_2 inhalation in accordance with the Animal Welfare Act as amended by the Public Health Service Policy. The rats were divided into 6 groups.

Group I—Five rats were used as the control group. On day 0, they were given halothane anesthesia, but no operative procedure was performed. On day 30, the animals were euthanized, blood was drawn, and testicles were harvested for histologic examination.

Group II—Five rats served as a sham control. On day 0, under halothane anesthesia, the left vas deferens was exposed but not divided. On day 30, they were euthanized, blood was drawn for antibody studies, and testicles were harvested for histologic examination.

Group III—Ten rats, on day 0, under halothane anesthetic, underwent a left vasectomy. On day 1, the animals were euthanized, blood was drawn, and testicles were harvested for histologic examination.

Group IV—Ten rats, on day 0, under halothane anesthetic, had a left vasectomy accomplished. On day 7, the animals were euthanized, blood was drawn, and testicles were harvested for histologic examination.

Group V—Ten rats, on day 0, under halothane anesthetic, had a left vasectomy performed, as above. On day 15, the animals were euthanized, blood was drawn, and testicles were harvested for histologic examination.

Group VI—Ten rats, on day 0, under halothane anesthetic, underwent a left vasectomy. On day 30, the animals were euthanized, blood was drawn, and testicles were harvested for histologic examination.

Histologic Examination

Testicles were fixed in Bouin solution and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin and examined by a single examiner, blinded to the coding of the specimens' origin. The testicles were evaluated histologically with respect to the following characteristics: 1) seminiferous tubular diameter (measured with a calibrated ocular micrometer); 2) morphology and progression of maturation of the germinal epithelium; and 3) morphology of the tunica propria and interstitial components, specifically to determine whether fibrosis, hyalinization, or an inflammatory infiltrate could be identified. When abnormalities were recognized, they were graded ''rare'' (confined only to isolated tubules), ''focal'' (confined to a discrete field and in more than 1 tubule), or ''diffuse'' (present in all tubules uniformly).

Immunologic Assays

Serum was withdrawn from the blood samples and immediately frozen at −70°C. The specimens, labeled with a random 2-letter code, were shipped in dry ice overnight to Oregon Health Sciences University, in Portland, Ore, and antisperm antibody levels were determined by an immunobead assay. This test was accomplished by adding 1 mL of test serum to a test tube with 0.3 mL of medium 199. To this suspension, 0.5 mL of purified rat sperm was added. The mixture was incubated at 37°C for 30 minutes. After incubation, 10 μL of serum/sperm suspension was mixed with 10 μL of goat anti-rat IgG-coated Sepharose beads (Zymed Laboratories, South San Francisco, Calif). The density of bound antibodies was approximated by counting the number of motile sperm bound per

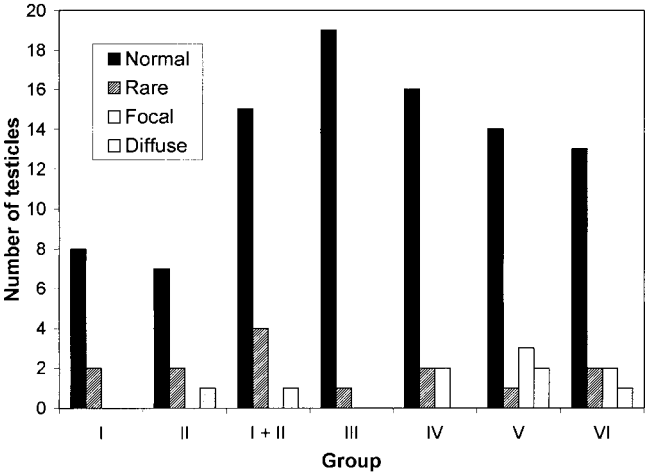

Figure 1. Degree of histologic damage judged by seminiferous tubular diameters, morphology of the germinal epithelium, and integrity of the interstitial compartments. The abnormalities, if present, were graded ''rare'' (confined only to isolated tubules), ''focal'' (confined to a discrete field but in more than just 1 tubule), or ''diffuse'' (present in all tubules uniformly). Key: Group I = 30-day control; group II = 30-day sham control; group I + II = combined 30-day control; group III = 1-day experimental; group IV = 7-day experimental; group V = 15-day experimental; and group VI = 30-day experimental.

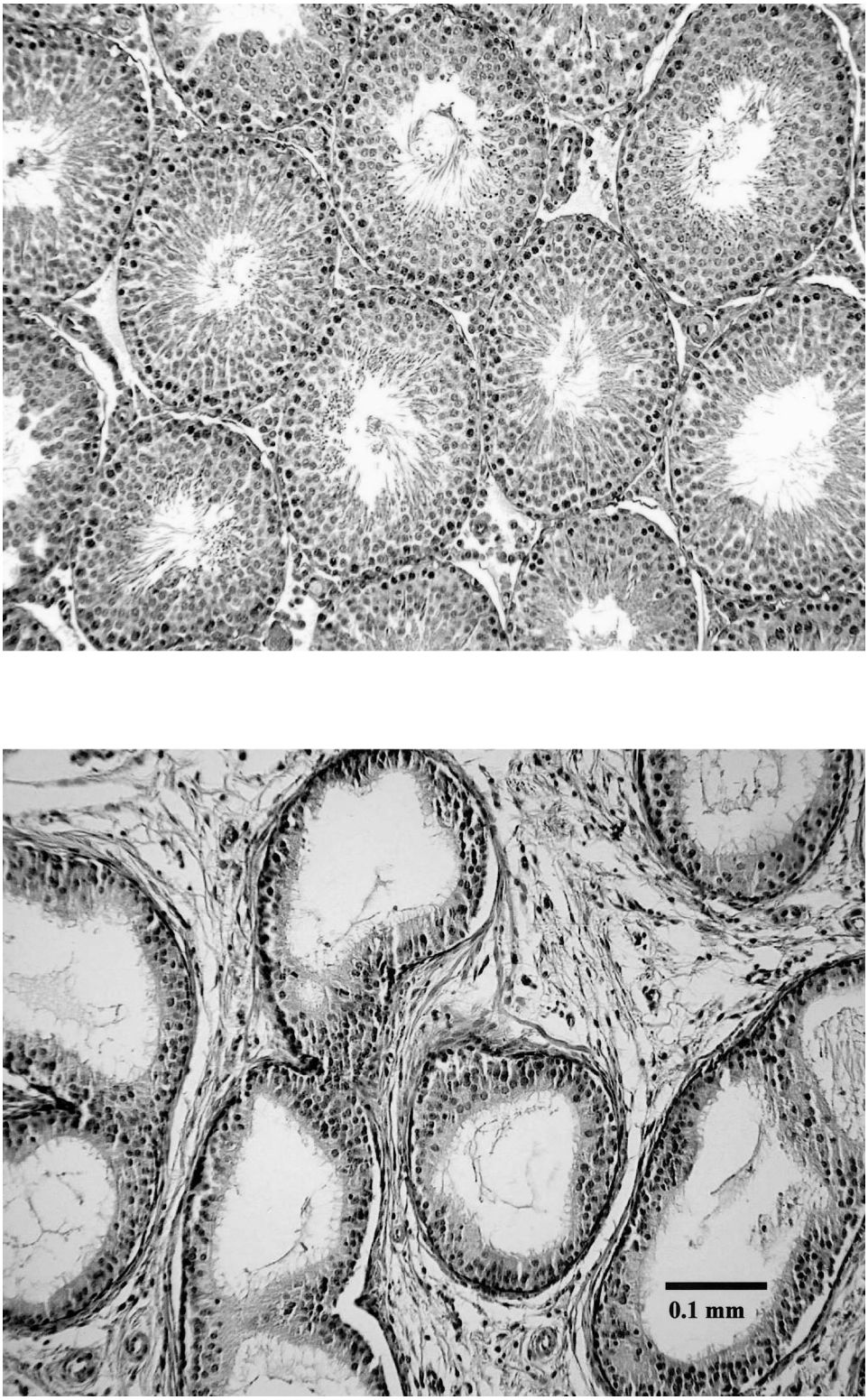

Figure 2. Comparison between a normal testis (top) and an altered testis with ''diffuse'' abnormalities seen in a small number of animals (bottom). Both specimens were obtained from rats following unilateral vasectomy and were stained with hematoxylin and eosin.

bead under the microscope at high power. This test was repeated for each specimen, and the 2 results were averaged.

Statistical analyses were performed by the Division of Biostatistics, Washington University School of Medicine, and are shown in Tables 1 and 2.

Results

Histologic Examination

There was no statistically significant difference in histologic examination between any of the groups. One testicle in group VI was deemed to have undergone change secondary to infection, and this animal was excluded from study. These results are shown in Figure 1. Sample photomicrographs are provided in Figure 2 in order to compare normal histology with the focal changes observed in some specimens.

Immunologic Evaluation

There was no statistically significant difference comparing groups I and II (the control animals) to the following: group III (animals euthanized at 1 day), *P* equals .21; group IV (animals euthanized at 7 days), *P* equals .50; or group V (animals euthanized at 15 days), *P* equals .44. There was, however, a statistically significant increase in immunobead antibody binding in group VI (animals euthanized at 30 days), *P* equals .02. These results are depicted in Table 1 (mean percentage binding) and Table 2 (percentage binding categorized as either a normal or positive result).

Antibody binding was assessed by a single investigator at the Oregon Health Sciences University who was blinded as to the coding of the specimens' origin. In that laboratory, greater than 40% binding is considered a positive result. When comparing the groups as to the number of animals with greater than 40% binding as a positive result and less than 40% as normal, the evaluation was as follows: comparing groups I and II, the controls, there was no difference compared to group III (animals euthanized at 1 day) *P* equals .35; group IV (animals euthanized at 7 days) *P* equals 1.00; or group V (animals euthanized at 15 days), *P* equals 1.00. There was a statistically significant difference between the control group and group VI (animals euthanized at 30 days), *P* equals .03.

No correlation, either positive or negative, between the presence of testicular abnormalities and immunobead binding assays was evident in individual animals.

Discussion

Unilateral injury to the vas deferens has been associated with damage to the contralateral testicle, and this damage is thought to be mediated through antisperm autoantibodies. It should be noted that a direct causal relationship has not yet been proven, and the issue remains somewhat controversial. It has previously been established that these antibodies do not develop until rats have become reproductively mature (Flickinger et al, 2000).

In this study, the immunobead assay we used did not detect significant levels of antisperm antibodies until after 15 days. Other studies have shown a response as early as 1 week after bilateral vasectomy in the Lewis rat model using the enzyme-linked immunosorbent assay method (Herr et al, 1987). It is unclear whether the cause for these differing results can be attributed to the experimental design (ie, unilateral vs bilateral vasectomy) or to the sensitivity of the assay method itself. When the immunologic results are examined in conjunction with the histologic data, they appear to indicate that the onset of the antibody response precedes any demonstrable histologic damage to the testicle. There was no statistically significant histologic difference between any of the groups, while at the same time, there was a significant difference in immunobead antibody binding in the 30-day group ($P = .02$).

The relationship between immunologic response, specific sperm antigens, and infertility has yet to be clarified in humans, but experiments in the rat model may help us better understand immunologic infertility in men. It may be possible to infer that human antisperm antibody production will also precede histologic testicular damage, and further, that the development of human antisperm antibodies may take weeks. In cases of suspected or known ductal injury, the monitoring of antisperm antibody levels could possibly enable testicular damage to be predicted prior to its development and thus be avoided.

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